REMARKS

In a Final Office Action mailed May 18, 2007, the Examiner in charge of the above-noted application rejected Claims 13, and 15-24 under 35 USC 103(a) as unpatentable. Claims 1, 3-13 and 15-25 are pending in the application. Claims 1, 3-12 and 25 are withdrawn from consideration as relating to non-elected subject matter. Applicants respond below to the issues presented in the Office Action. In view of the claim amendments and arguments presented herein, applicants respectfully request reconsideration of the merits of this application.

Claim Amendments

Claim 13 is amended to clarify that the mammalian cells are human embryonic stem (ES) cells by including the limitation of Claim 15. As such, Claim 15 is canceled. Likewise, withdrawn method Claims 1 and 25 are amended to include the human ES cells of Claim 3. As such, Claim 3 is also canceled. These withdrawn claims are amended under Ochiai/Brouwer to prepare the non-elected Claims for rejoinder, once the product claims are found allowable. (See, *In re Ochiai*, 71 F. 3d 1565, 37 USPQ2d 1127 (Fed. Cir. 1995) and *In re Brouwer*, F.3d, 37 USPQ2d 1663 (Fed. Cir. 1996)).

New Claims 26-27, which read on the elected group and Claim 28, which is drawn to a non-elected, but rejoinable group are added to define the undifferentiated human stem cells by cell surface markers. Claims 27-27 mirror elected Claim 13. New Claim 28 mirrors Claim 1. Support is found, for example, at Fig. 2 (paragraph 20) and paragraphs 27, 28, 44, 45, 46, and 48. No new matter is introduced with the above-noted claim amendments. Based on these claim amendments and the remarks below, it is believed that all of the claims are in condition for allowance.

Claim Rejections - 35 USC §103

Claims 13 and 15-24 are rejected under 35 USC 103(a) as unpatentable over Flexcell (see attached Flexcell International Corporation Web Page) and Banes (US Pat. No. 6,037,141) in view of Xu et al. (2001) and Thies (US Pub. App. 2003/0109038).

As a preliminary matter applicants respectfully submit that there is no logical tie between the cited documents and the purported "obviousness" that the application of strain to undifferentiated human stem cells maintains them in an undifferentiated state. Notably,

applicant's findings were quite surprising and different from their expected result. In fact, the applicants have found that their data often surprise other scientists in the field who also expected strain to stimulate differentiation, as it does in many types of primary cells. To applicants' knowledge, there are no reported instances of strain repressing differentiation in any cell type.

To illustrate that applicants' understanding is also well recognized in the literature, as evidence for the record, applicants submit the following documents discussed below in a supplemental Information Disclosure Statement (the documents will be sent in a separate mailing). Specifically, the literature provides that strain induces differentiation or a phenotype associated with differentiation in primary cell types or stem cells (e.g., mesenchymal stem cell), not maintaining cells in an undifferentiated status.

Notably, mechanical stimulation is reported to induce differentiation in human osteoblasts cultured on metallic biomaterials (Di Palma F., et al. 2003, Physiological strains induce differentiation in human osteoblasts cultured on orthopaedic biomaterial. *Biomaterials* 24(18): 3139-3151). Mechanical stress induces the selective differentiation of mesenchymal progenitor cells from the bone marrow *in vitro*, even in the absence of ligament-specific exogenous growth and differentiation factors (Altman et al., 2002. Cell differentiation by Mechanical stress. *FASEB Journal* 16(2): 270-272). Also, recently, cyclic uniaxial strain, but not cyclic equiaxial strain, has been reported to upregulate the expression of smooth muscle cell (SMC) markers in mesenchymal stem cells (Park et al., 2004, Differential effects of equiaxial and uniaxial strain on mesenchymal stem cells. *Biotechnology and Bioengineering* 88(3): 359-368). Mechanical stretch applied using a Flexercell Strain Unit induced differentiation and maturation of fetal alveolar epithelial cells, presumably by simulating fetal breathing movement (Sanchez- Esteban et al., 2000, Mechanical stretch promotes alveolar epithelial type II cell differentiation. *J Appl Physiol* 91(2): 589-595).

Further, cyclic mechanical stretch possessed dual effects on vascular smooth cell phenotype characteristics by 1) potentiating proliferation, an attribute of a dedifferentiated phenotype, and 2) increasing expression of h-caldesmon, considered a marker of differentiated smooth muscle cells (Birukov et al., 1995, Stretch affects phenotype and proliferation of vascular smooth muscle cells. *Mol. Cell Biochem.* ; 144(2): 131-139). Mechanical stretching is also a potent stimulator of gene expression and signal transduction in endothelial cells, vascular smooth muscle cells and stretched osteocytes (Chien et al., 1998, Effects of mechanical forces on signal

transduction and gene expression in endothelial cells. *Hypertension*; 31(1 Pt 2): 162-169; Zou et al., 1995, Signal transduction in arteriosclerosis: mechanical stress-activated MAP kinases in vascular smooth muscle cells. *Int J Mol Med* 1(5): 827-834; and Kamata et al., 1998, Mechanotransduction in stretched osteocytes- Temporal expression of immediate early and other genes. *Biochem Biophys Res Commun* 246 (2): 404-408). Pulsatile mechanical stretch induced rapid secretion of vascular endothelial growth factor (VEGF) by cultured rat cardiac myocytes, which may play a role in ameliorating the relative myocardial hypoxia (Seko et al., 1999, Pulsatile stretch stimulates vascular endothelial growth factor (VEGF) secretion by cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 254(2): 462-465). Clearly, based on the literature and the general understanding in the stem cell industry, it was not obvious that strain represses differentiation. On the contrary, the literature states the exact opposite.

Turning now to the specific rejection, the Examiner alleges that

> "the web page of Flexcell teaches a cell culture apparatus with BioFlex culture plates, which can provide periodic strain by applying vacuum pressure, and oscillatory strain." (See, pg. 3, last para. of current Office Action).

Further, on page 4 of the current Action, the Examiner alleges that

> "Banes teach a culture compression device made from a flexible membrane, wherein the cell culture can be compressed to introduce mechanical strain with cycles of pressure pulses (periodic strain)."

The Examiner asserts that Banes teaches that force can be applied by the Flexcell strain unit taught by the web page (see Abstract and column 6, lines 54-67 of Banes). Applicants respectfully disagree.

Applicants submit that the use of Flexcell and/or Banes, as a primary reference to render the claims obvious is fundamentally flawed. The Flexcell webpage simply discloses a picture of an apparatus (FX-3000) consisting of a screen monitor, keyboard, hard drive and cell culture plates. The webpage also shows a single cell at rest and under stress, presumably stretched in culture with the FX-3000 system.

Further, Banes discloses a generic cell culture compression device, where the sample support is compressed against a "stop member" thereby compressing a cell culture retained on the support. Banes discloses a culture "compression" assembly for culturing cells under compressive loads. Banes' device applies pressure to the cell culture by compressing or pushing

down on the cells through delivering force to the cells in pounds (lbs.) vs. applied pressure in pounds per square inch (psi). (*See Banes*, col. 7, lines 1-11). There is a difference between compressing cells vs. stretching cells. Banes does not stretch cells in culture.

Moreover, the purpose of Bane's device is to prepare already differentiated cells for (1) implantation into a patient, such as by conditioning explants of cartilage to withstand the rigors of the *in vivo* environment in a joint, or (2) studying the impact of compressive load placement upon specialized cell cultures. Banes does not disclose undifferentiated human stem cells, much less attempt to maintain human ES cells in an undifferentiated state by stretching the matrix and the cells. The idea of stretching human ES cells while proliferating on a flexible solid porous matrix to maintain them in an undifferentiated state was an unexpected discovery made by the applicants and disclosed exclusively in the instant application.

Neither Flexcell nor Banes disclose (1) undifferentiated human stem cells or methods to culture such undifferentiated cells, (2) a flexible solid porous matrix, (3) stretching the matrix and the human ES cells thereon using periodic strain; (4) human ES cell proliferation and (5) the main purpose behind applicants' invention, which is to reduce human ES cell differentiation and maintain ES cells in an undifferentiated state. Thus, these primary references cannot be used to render the claims obvious. Further, none of the remaining documents cited by the Examiner, Xu et al. or Thies, discussed herein below, are sufficient to cure the deficiencies of Flexcell and Banes.

In relation to Xu et al., the Examiner alleges that it teaches a culture of human ES cells grown on Matrigel™ without conditioned media or feeder cells, which inhibits differentiation of human ES cells. The Examiner concludes that it would have been obvious for one of skill in the art at the time the invention was made to use the apparatus of Flexcell to grow human ES cells as taught by Xu et al. Further, the Office Action provides that

> "[T]he motivation of using the apparatus of Flexcell in human ES cell culture is provided by Banes that chondrocytes, cells for cartilage transplantation, would be advantageous when they are grown under compression.... Since hES cells can be differentiated into chondrocytes which constitute cartilage as supported by Thies, a person of ordinary skill in the art would have envisaged growing hES cells using an apparatus of Flexcell and/or Banes to obtain a plurality of cells, which would be differentiated into chondrocytes prior to transplantation." (See bottom of pg. 4).

In response and as a preliminary matter, applicants wish to make clear that a person working in the stem cell field would readily recognize that undifferentiated human stem cells are different from the "differentiated" adult cells as taught by Banes. Human ES cells are primary undifferentiated (uncommitted) cells. Human ES cells are unlike any specific adult cell. However, they have the ability to specialize and form any adult cell. Because undifferentiated human ES cells can proliferate indefinitely in culture, they could potentially provide an unlimited source of specific, clinically important differentiated adult cells such as bone, nerve, muscle, liver or blood cells. In contrast, the cells taught by Banes are differentiated adult cells committed to a particular cell lineage, such as chondrocytes.

In addition to the differences mentioned above, undifferentiated human ES cells and differentiated cells differ in their cell surface markers. They also differ in the conditions and the factors used in culturing and expanding the cells. Indeed, stem cell researchers, in particular those working on human ES cells, go to great lengths to maintain these cells in a proliferating and undifferentiated state. This is not a trivial task and not obvious.

Because of the wide array of differences between human ES cells and a differentiated cell, one of ordinary skill in that art would not look to a device that is used to culture terminally differentiated cells, like chondrocytes, to help maintain human ES cells in an undifferentiated state. Further, there is no expectation of success when culture conditions used with terminally differentiated cells, chondrocytes, are substituted for undifferentiated human stem cells. This is particularly true when the purpose of the claimed invention is to maintain undifferentiated human stem cells in a proliferative yet undifferentiated state.

Having established the difference between undifferentiated human stem cells and differentiated cells, applicants now turn to the secondary references. Xu et al. disclose culturing human ES cells to maintain them in an undifferentiated state. However, Xu et al. do not (1) disclose stretching of a matrix or cells thereon by applying an effective amount of periodic strain on the flexible matrix or (2) provide any motivation as to why such stretching would be beneficial for maintaining human ES cells in an undifferentiated state. Applicants believe that there would be no motivation to combine the cited documents to arrive at the claims, as it appears the human ES cell culture system of Xu et al. is suitable for scale up production, without the need to stretch the cells by applying periodic strain. (*See*, pg. 971, col. 1, last sentence of Abstract).

Likewise, at best, Thies does nothing more than to *teach away* from maintaining human ES cells in an undifferentiated state as they proliferate. Thies discloses a differentiated cell population, chondrocytes, and methods for obtaining it. Specifically, Thies discloses a system for obtaining cells of the chondrocyte lineage by differentiating primate pluripotent stem cells. The process involves culturing the cells as a micromass or other aggregate form in a cocktail of differentiation agents that facilitates outgrowth of the desired cell type. Progeny are capable of synthesizing Type II collagen or aggrecan, or other products that are characteristic of the chondrocyte lineage. This concept is also clearly embodied in the title of the invention "Chondrocyte precursors derived from human embryonic stem cells." As such, nowhere in the current Office Action does it explain why one would have been motivated to combine Flexcell, Banes, Xu et al., and Thies absent a teaching from applicants' own disclosure that stretching by applying periodic strain results in the benefit of reduced differentiation of human ES cells. Therefore, applicants believe that the Examiner has improperly cited the combination of documents and that a *prima facie* case of obviousness was not made.

Applicants have made a diligent effort to place the claims in condition for allowance. However, should there remain unresolved issues that require adverse action, it is respectfully requested that the Examiner telephone applicants' attorney at the number listed below so that such issues may be resolved as expeditiously as possible. For the reasons stated above this application is now considered to be in condition for allowance and such action is earnestly solicited.

Fees

A Request for Continuing Examination (RCE) accompanies this response so that it will be deemed to have been timely filed. No extension of time is believed due, but should any extension be due, in this or any subsequent response, please consider this to be a petition for the appropriate extension and a request to charge the extension fee to Deposit Account No. 17-0055. No additional fees are believed due; however, if any fees are due, in this or any subsequent response, please charge Deposit Account 17-0055.

Respectfully submitted,

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